bio-protocol Preprint

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Primary osteoclast culture

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An abbreviated version of this protocol was published in eLIFE in Aug 2020

Irisin directly stimulates osteoclastogenesis and bone resorption in vitro and in vivo

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Detailed protocol

Primary Osteoclast Differentiation Culture from Murine Bone Marrow Progenitors

Rosen Lab – Edited by EGE 12/01/21

Bone Marrow Isolation

Materials

-Mice (n = 3 littermates) around 8 weeks of age
-Sterile scissors & tweezers
-1mL syringes & 25G needles
-1.5mL microcentrifuge tubes
-Kimwipes
-Trypan Blue & Hemocytometer
-70% EtOH
-Sterile chilled PBS
-Sterile petri dishes
-15 & 50mL conical tubes
-70µm cell strainers
-Culture media: αMEM+10%FBS/1% Pen-Strep

Limb Collection (chemistry hood)

1. Sac animals via CO_2 followed by cervical dislocation – spray thoroughly with 70% EtOH
2. Pinching skin of lower back, make cut with scissors
3. Peel back skin to widen incision completely around body, then pull down off limbs to feet
4. Cut just below ankle joint to remove feet and attached skin
5. Place index finger at back of ileac crest and apply pressure to front with thumb to dislocate hip
6. Cut through muscle around exposed femoral head to free limb, place in sterile cold PBS

Marrow Collection (tissue culture hood)

1. Spray down hood surfaces and all equipment with 70% EtOH
2. Manually remove muscle from limbs with Kimwipe, place bones in petri dish with sterile PBS
3. Prepare microcentrifuge tubes with 200µL culture media and trimmed 200µL pipette tip
4. Isolate femur and tibia by cutting below femoral head, above/below knee, and below ankle
5. Place 2 femur and 2 tibia per microcentrifuge tube tip insert, packed in securely
6. Perform quick spin (15s @ 13Krpm) to remove marrow from the femur and tibia
7. Remove bones from pipette tip with tweezers, flush remaining marrow from tip with pipette
8. Combine and transfer all marrow samples in group into 15mL conical tube
9. Add 10mL culture media, pipet to homogenize, filter via 70µm cell strainer into 50mL conical
10. Add 20mL culture media and plate into one T150 or two T75 flasks

Osteoclast Differentiation

After 48hrs of culture, flasks now contain adherent MSCs (osteoblast & adipocyte progenitors) on surface and non-adherent HSCs (osteoclast progenitors) in culture media

RANKL Stock
10µg RANKL (PeproTech 310-01)
+400µl sterile water
> Orbital shake on ice for 30 min
> Aliquot 50µl, store at -80°C

M-CSF Stock
10µg m-CSF (PeproTech 315-02)
+400uL sterile water
> Orbital shake on ice for 30 min
> Aliquot 25µl, store at -80°C

Osteoclast Differentiation Media (OCL-DM)

Per 1mL culture media (pre-filtered):
+4µl RANKL
+1.2µl M-CSF
> Make desired volume & use fresh, or store @ 4°C for up to a week

1. Collect culture media with non-adherent cells from bone marrow flasks, spin down (1.5Krpm for 10min), resuspend in 1mL OCL-DM, count, and plate in osteoclast media at $1.56x10^5$ cells/cm^2 (96-well plate: 50K cell/well, 12-well plate: 600K cell/well)
    - Osteoclasts can be cultured on tissue culture treated plastic for TRAP staining, or desired matrix surface (Corning OsteoAssay 3988, Lonza OsteoLyse PA-1500, IDS Bone Slices DT-1BON1000-96) for resorption pit staining
2. Culture in OCL-DM, replacing media every other day, observing for first appearance of fused cells within 3-5 days, widespread fusion of large osteoclasts should occur by 7-10 days
3. Wash wells with PBS and fix in 10% formalin, then stain for mature osteoclasts with TRAP kit (Sigma Aldrich 42010102)
4. Image wells, counting blinded images for TRAP-positive cells with >3 nuclei to quantify mature osteoclasts/well
5. For resorption cultures, follow established protocols for respective assay surface to quantify resorption via CTX release or resorption pit area (Von Kossa stain for OsteoAssay, included ELISA for OsteoLyse, Crosslaps CTX Assay and/or Toluidine Blue pit staining for bone slices)

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Estell, E. and Rosen, C. (2022). Primary osteoclast culture. Bio-protocol Preprint. bio-protocol.org/prep1571.
2. Estell, E. G., Le, P. T., Vegting, Y., Kim, H., Wrann, C., Bouxsein, M. L., Nagano, K., Baron, R., Spiegelman, B. M. and Rosen, C. J.(2020). Irisin directly stimulates osteoclastogenesis and bone resorption in vitro and in vivo. eLIFE. DOI: 10.7554/eLife.58172